DIFFERENTIAL TOLERANCE OF HAWAIIAN SUGARCANE VARIETIES TO DIURON, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

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DIFFERENTIAL TOLERANCE OF HAWAIIAN SUGARCANE VARIETIES TO DIURON, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

By Robert Vernon Osgood

A dissertation submitted to the Graduate Division of the University of Hawaii in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Abstract

Hawaiian sugarcane varieties, which are interspecific hybrids within the genus Saccharum, were found to differ substantially in their tolerance to diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, and ametryne, 2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine. Metabolism, uptake and distribution of diuron were studied as possible mechanisms of the differential varietal response.

In a field experiment diuron and ametryne were applied at 9 lb./acre (4 lb./acre pre-emergence + 5 lb./acre post-emergence) to three varieties of sugarcane which were reported to be either susceptible (H 53-263), intermediately susceptible (H 57-5174), or tolerant (H 50-7209) to herbicides. Using growth in height and diameter as criteria of phytotoxicity, variety H 53-263 was found to be susceptible to both diuron and ametryne, while varieties H 50-7209 and H 57-5174 were found to be tolerant. Tillering was reduced in all varieties; however, reduction was greatest in variety H 53-263.

In nutrient solution the concentrations of diuron which brought about a 50% reduction in the fresh weight of variety H 53-263 and H 50-7209 were 0.35 and 1.6 ppm, respectively. Monomethyldiuron, a diuron metabolite, was found to be about 2.5 times less toxic to H 53-263 than diuron.

Diuron-^{14}C was found to be degraded in both the resistant variety, H 50-7209, and the susceptible variety, H 53-263. The primary metabolites were monomethyldiuron, 1-(3,4-dichlorophenyl)-3-methylurea, and demethylated diuron, 3,4-dichlorophenylurea. Degradation of diuron was more extensive in the resistant variety, H 50-7209, than in the susceptible variety H 53-263.

In an uptake and translocation study, both the tolerant and susceptible varieties removed approximately 80% of carbonyl-labeled diuron from nutrient solution within 9 days after treatment. The distribution of radioactivity 14 days after treatment was almost identical in both varieties when calculated on a total activity basis; however, when the distribution data were calculated on the basis of activity per unit of dry weight, there was a greater concentration of radioactivity in the younger leaves of the susceptible variety, H 53-263, than in the resistant variety, H 50-7209. Conversely, there was a greater concentration of radioactivity in the

roots of H 50-7209 than in H 53-263.

The differential varietal response to diuron exhibited by varieties H 53-263 and H 50-7209 is believed to be at least partially explained by differential metabolism and translocation.

DIFFERENTIAL TOLERANCE OF HAWAIIAN SUGARCANE VARIETIES TO DIURON, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

INTRODUCTION

Hawaiian sugarcane varieties, which are interspecific hybrids within the genus Saccharum, have been observed to differ substantially in their tolerance to both pre- and post-emergence applications of substituted phenylurea and triazine herbicides. Variety H 53-263, which presently occupies about 21,000 unirrigated acres on the islands of Hawaii and Kauai, has been especially susceptible to applications of diuron; variety H 50-7209, which is grown on the majority of the irrigated acreage, has been notably resistant to diuron.

On the Hilo coast of Hawaii and on windward Kauai, where diuron is presently the most efficient single pre-emergence herbicide registered for use in sugarcane, the susceptible variety H 53-263 occupies large acreage and, as a result, the plantations have been forced to modify their standard herbicide practices in order to avoid phytotoxicity to the crop.

The purpose of the present study was to further define the variation in herbicide toxicity between resistant and susceptible sugarcane varieties and to study possible causes for the phenomenon, such as differential uptake, translocation, and metabolism.

LITERATURE REVIEW

Varietal Tolerance of Sugarcane to Herbicides.

Sugarcane varieties in Hawaii and other parts of the world have been observed to differ substantially in their tolerance to foliar and soil-applied herbicides. Nolla (1950), in an extensive field study in Puerto Rico, found that sugarcane varieties were differentially susceptible to 2,4-D; he reported 5 varieties tolerant, 2 slightly susceptible, 2 moderately susceptible, and 4 susceptible. Sugarcane under 2 months old was protected from 2,4-D injury by closely united leaf sheaths which acted as a barrier toward entry of 2,4-D in the meristematic region; however, several clones were not injured when 2,4-D was applied directly to the intercalary meristem. Two kinds of resistance were postulated: 1) escape, as shown by young cane, and 2) physiological resistance.

Denison (1960) reported the effects of 2,4-D, monuron, diuron, simazine, dalapon, and TCA on 8 varieties of Hawaiian sugarcane. The varietal tolerance of cane to diuron applied twice at 5 lb. a.i./acre was as follows: H 39-5803 -- heavy injury; (H 37-1933, H 49-5) -- moderate injury; H 49-104 -- slight to moderate injury; (H 49-3533, H 44-3098) -- slight

injury; (H 50-7209, H 39-7028) -- no injury. Simazine was the least phytotoxic of the herbicides studied while dalapon and 2,4-D (ester) were the most phytotoxic.

Wiemer (1963) reported on the effects of 8 herbicides (monuron, diuron, linuron, atrazine, prometone, dalapon, amitrole, and 2,4-D) on 8 Hawaiian sugarcane varieties (H 37-1933, H 38-2915, H 39-5803, H 44-3098, H 49-5, H 49-3533, H 50-7209, and H 50-2036). Although the experiment was carried out in 1961, four of the herbicides (diuron, atrazine, dalapon, and 2,4-D) are still industry standards, and three of the varieties (H 50-7209, H 49-5, and H 49-3533) occupy over 50% of the sugarcane acreage in Hawaii. Wiemer applied the herbicides by 3 methods (over exposed seed, over covered seed, and over cane) and the effects of the herbicides were measured in terms of germination, harvest weights, and cane effect (visual injury). When germination counts were made, varieties H 44-3090, H 49-3533, and H 50-7209 were rated resistant; varieties H 39-5803, H 38-2915, H 49-5, and H 50-2036 were intermediate; H 37-1933 was susceptible.

Harvest data (green weight at 6 1/3 months) showed variety H 49-3533 to be resistant; varieties H 44-3098, H 50-7209, H 38-2915, H 50-2036, and H 49-5 were intermediate; varieties

H 37-1933 and H 39-5803 were susceptible. Diuron and linuron were the least damaging herbicides, followed closely by atrazine, monuron, prometone, and 2,4-D. Dalapon and amitrole were the most damaging.

Visual cane effect gradings showed varieties H 44-3098 and H 50-7209 to be resistant. The other varieties are listed in the order of increasing susceptibility: H 49-3533, H 49-5, H 50-2036, H 38-2915, H 37-1933, and H 39-5803. The order of increasing effect of herbicides based on cane effect was as follows: monuron, atrazine, diuron, 2,4-D, prometone, linuron, dalapon, and amitrole. The post-emergence over-cane applications were the most damaging, and normal pre-emergence applications were the least damaging.

Variety x herbicide interactions were significant for the germination and harvest-weight data, indicating that the cane varieties studied were differentially susceptible to the herbicide treatments.

Hilton et al. (1965) and Hilton and Nomura (1966) reported that commercial Hawaiian sugarcane varieties were differentially tolerant to 1 ppm diuron in nutrient solution. Varieties H 49-3533, H 50-7209 (butt seed), H 54-775, and H 57-5174 were tolerant; H 39-7028, H 44-3098, H 50-7209 (top seed), H 50-5217, H 50-2036, and H 49-5 were intermediately

tolerant; H 37-1933 and H 53-263 were susceptible. Symptoms of diuron injury were manifested in reduced growth and tillering and not the typical phenylurea chlorosis which is observed in the field. All replications of variety H 53-263 were either dead or dying at the termination of the experiment.

When the above varieties were analyzed, diuron residue (measured as 3,4-dichloroaniline) increased slightly with increasing susceptibility; however, it was concluded that differences were not great enough to account for the very large differences in growth; e.g., variety H 49-3533 showed only slight stunting and contained 9 ppm diuron in the dried leaf tissue; whereas variety H 53-263 was killed and contained 11 ppm diuron. Since diuron was measured as 3,4-dichloroaniline, it is probable that diuron metabolites, such as monomethyldiuron or the demethylated derivative, were also measured. The differential varietal response may have resulted from quantitative differences in diuron metabolism.

Further evidence for differential varietal tolerance in sugarcane was reported from Louisiana by Millhollon and Matherne (1968). They found varieties C.P.44-101 and C.P.52-68 to be severely injured by diuron while variety N.Co.310 was unaffected. Conversely, N.Co.310 was more

severely injured by dalapon than other varieties. A combination of Na-TCA and 2,4-D at 10.6 and 3 lb./acre reduced the yield of N.Co.310 in one test, but did not reduce the yield of C.P.44-101 or C.P.52-68 in either test. All varieties were tolerant of fenac applied at 4 to 12 lb./acre. Diuron at 9 lb./acre injured C.P.44-101 and C.P.52-68, when applied to the foliage or to the soil and reduced the yield by 48% and 32%, respectively. Variety N.Co.310 tolerated both 9 and 12 lb./acre of diuron with no significant decrease in yield or visual injury. N.Co.310 was, however, severely injured by dalapon at 8 lb./acre in one test and by 5.2 and 10.4 lb./acre in another test.

Leading commercial varieties in Mauritius have not been observed to be differentially susceptible to diuron (Rochecouste, 1963), although differences in susceptibility to dalapon and paraquat have been noted (Rochecouste 1962, 1967). Variety Ebène 137 was tolerant of paraquat while Ebène 50/47 was moderately susceptible. Rochecouste (1964) found that one variety was susceptible to both bromacil and isocil while 3 other varieties were fairly tolerant.

Substituted Phenylurea Herbicides

Mode of Action

The substituted phenylurea herbicides are highly

effective inhibitors of photosynthesis. These compounds apparently interfere with the return flow of electrons to the ground state after activation by light in Photosystem II (the oxygen evolution system of photosynthesis). Diuron is one of the most powerful inhibitors of Photosystem II and has been used extensively in photosynthesis experiments. Van Overbeek (1964) explained that the substituted phenylurea herbicides are phytotoxic primarily because they predispose plant tissue to damage by light.

Sweetser (1962) reported that the activity of monuron, 3-(p-chlorophenyl)-1,1-dimethylurea, and other substituted phenylureas, was dependent on the ability of the herbicide to react photochemically with flavine mononucleotide (FMN). Upon reaction with FMN the phenylurea herbicides were inactivated. Addition of a 1% solution of FMN to pinto bean plants provided protection from monuron.

Phytoactivity

Christoph and Fisk (1954) reported that monuron toxicity in barley was manifested by loss of turgor, chlorosis, and progressive dying of older leaves. Mitosis was retarded in apical meristems. Both shoot and root reduction were noted when topical applications of CMU were made to tomato internodes. Muzik et al. (1954) grew excised velvet bean roots

for three months in nutrient solution containing monuron at several concentrations; they recorded only slight injury at 10 ppm but severe injury from 25, 50, 100, and 250 ppm. Since 10 ppm is sufficient to kill intact velvet beans, they concluded that the primary site of monuron activity was in the tops.

In sugarcane, diuron symptoms usually appear as a temporary chlorosis on the older leaves. However, in some instances, the chlorosis is followed by die-back of the older leaves, reduced tillering, necrosis of the spindle leaves, and, in very severe cases, death of the plant. Phytotoxicity may also occur as stunting without chlorotic or necrotic symptoms. Stunting of the root system has also been observed; however, it is not known if the phenylureas act directly on the sugarcane root tissue.

Absorption and Translocation

Minshall (1954) found that when monuron (CMU) was applied to bean and tomato roots, it was rapidly carried to the tops, where it was accumulated until a lethal concentration was reached. Roots remained alive for several days after the tops were killed. He concluded that the primary action of monuron occurred in the leaves and that diuron symptoms could be delayed by shielding leaves from light.

Fang et al. (1955) found that when monuron was applied to the leaves of bean plants, it was rapidly absorbed and translocated within the treated leaf, but movement to other leaves was restricted. Bayer and Yamaguchi (1965) showed that diuron moved acropetally in the transpiration stream of soybean, red kidney bean, and barley; movement did not occur in the phloem. Differential rates of absorption and translocation were not observed in the three species studied. The amount taken up and distributed increased with time. Addition of a surfactant did not alter the translocation of diuron. Rogers and Funderburk (1967) reported that cotoran, 3-(m-trifluoromethylphenyl)-1,1-dimethylurea, was not differentially absorbed by cotton (resistant) and cucumber (susceptible); however, differences in distribution and metabolism were noted. No basipetal translocation was observed when leaf applications were made. Haun and Peterson (1954) reported that monuron translocation was acropetal in both monocotyledonous and dicotyledonous plants. Muzik et al. (1954) showed that CMU (monuron) readily entered leaves, stems, and roots. Movement in the leaves was most rapid between the veins and appeared to be blocked by larger veins. Entry into roots was rapid and translocation was primarily toward the shoot apex in the transpiration stream.

Metabolism

When Fang et al. (1955) extracted ^{14}C-CMU-treated bean leaves in 80% ethanol and chromatographed the extracts, they found both CMU and an unknown "CMU-complex". The concentration of the "CMU-complex" increased with time at the expense of CMU, and at 12 hours after treatment 19% of the radioactivity was present as the "complex". They proposed that CMU was hydrolyzed with the subsequent formation of p-chloroaniline, dimethylamine, and radioactive carbon dioxide; however, no convincing evidence was presented to support such a breakdown scheme. The authors did not consider the possibility that demethylation might have occurred, yielding monomethylmonuron and demethylated monuron.

Smith and Sheets (1967) found that both monuron and diuron were metabolized in soybean, cotton, corn, and oats; however, there were large qualitative and quantitative differences in the formation of metabolites. A 10-fold difference in the toxicity of diuron to cotton (tolerant) and soybean (susceptible) was explained by a more complete degradation of diuron in the former. At 120 hours after treatment with carbonyl-labeled diuron, soybean leaves contained 57% phytotoxic compounds (diuron and its monomethyl derivative) while cotton contained only 20% (all present as

monomethyldiuron which is one-half as toxic as diuron). When ring-labeled ^{14}C monuron treatments were made, soybean contained 53% as monuron and monomethylmonuron residues, while cotton contained only 26%. Differences in toxicity to monuron and diuron among susceptible oat, soybean, and corn were related more to differential absorption than to differential metabolism.

Olney et al. (1968) reported that corn seedlings metabolized diuron to 3-(3,4-dichlorophenyl)-1-methylurea, 3,4-dichlorophenylurea, 3,4-dichloroaniline, and 3,4-dichlorobenzene. Two pathways of metabolism were proposed: one for applications to nutrient solution, and one for applications to leaves. In nutrient solution it was proposed that diuron was first demethylated with subsequent hydrolysis and formation of 3,4-dichloroaniline which was oxidized to form 3,4-dichloronitrobenzene. When applications were made to leaves, the expected metabolites were not detected. Either the metabolites were not formed in the leaves or they were in too small a quantity for detection. Several unidentified peaks were detected in extracts from treated leaves. Only small amounts of parent diuron were detected at 8 days, indicating that metabolism occurred, but along a different pathway from that proposed for the roots.

No diuron or degradation products were exuded into the nutrient solution.

Swanson and Swanson (1968a) described a method for studying the metabolism of the phenylurea herbicides, monuron and diuron, utilizing leaf discs of resistant and susceptible plants. They found that leaf tissue of phenylurea-resistant cotton and plantain (Plantago major L.) could readily metabolize both diuron and monuron. The phenylurea-susceptible plants, corn and soybean, could also metabolize the same herbicides, but to a more limited extent. They also reported quantitative and qualitative differences in degradation in resistant and susceptible species. Plantain and cotton leaf discs readily degraded diuron to monomethyldiuron, 1-(3,4-dichlorophenyl)-3-methylurea and further demethylated this compound to 3,4-dichlorophenylurea. In soybean leaf discs metabolism did not progress beyond the first demethylation, and in corn only trace amounts of monomethyldiuron were produced.

Smith and Sheets (1967) and Olney et al. (1968) reported that diuron metabolism was at least partially accomplished in the roots; but Swanson and Swanson (1968a) have shown that if diuron reaches the leaf tissue it is readily degraded, at least in resistant species.

Swanson and Swanson (1968b) reported that degradation of monuron was strongly inhibited by the insecticide carbaryl (1-naphthylmethylcarbamate). Carbaryl did not inhibit the degradation of monuron to monomethylmonuron, but inhibited the formation of demethylated monuron, i.e., the demethylation of monomethylmonuron. These data were collected by the leaf disc method described in Swanson and Swanson (1968a).

Dalton et al. (1966) showed that diuron was degraded in soil to the monomethyl and demethylated derivative with subsequent hydrolysis of the urea to form 3,4-dichloroaniline. They proposed that the 3,4-dichloroaniline was further degraded to carbon dioxide, ammonia, and chloride; however, evidence was not presented which could explain metabolism beyond 3,4-dichloroaniline.

MATERIALS AND METHODS

Phytotoxicity Studies

Field experiment. In order to test the hypothesis that Hawaiian sugarcane varieties are differentially susceptible to diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] and ametryne [2-(ethylamino)-4-(isopropylamino-6-(methylmercapto)-s-triazine], a factorial experiment was designed and installed with furrow irrigation in Field P-2 of the Poamoho Experimental Farm, University of Hawaii Agricultural Experiment Station. The study commenced July 17, 1967 and was terminated January 9, 1968.

The experiment was designed as a randomized complete block with a factorial arrangement of treatments (3 varieties x 3 treatments). Varieties H 50-7209, H 57-5174, and H 53-263 were treated with a split application of 9 lb/acre (active ingredient) of diuron and ametryne. Four lb of diuron and ametryne were broadcast overall in water at 40 gallons/acre 2 days after planting, and 5 lb of the same herbicides plus 0.1% X-77 surfactant were broadcast interline in 57 gallons/acre at 49 days after planting. Check plots were kept weed free by hoeing and spot applications of paraquat. There were 3 replications of each treatment.

The 800 sq. foot plots (40'long by 20' wide) were

planted with 32 three-eyed seedpieces spaced 5 feet apart in each of 4 lines. Only 1 eye was allowed to develop from each seedpiece. Data were taken from the center 6 stools of the middle 2 lines in each plot. Height data (ground to first visible dewlap) were taken at 46, 58, 71, 92, and 138 days after treatment. Diameter and tillering were recorded 138 and 188 days after treatment, respectively. Average values for the growth measurements were obtained for each plot and subjected to analysis of variance. Orthogonal single degree of freedom comparisons were computed when applicable.

Nutrient solution experiments. Three phytotoxicity experiments were conducted under controlled conditions in aerated nutrient solution. In each experiment plants of variety H 53-263 or H 50-7209 were established in 1 liter of nutrient solution and placed in a growth chamber set for the following conditions: day temperature 85°F, day humidity 65%, night temperature 75°F, night humidity 90%. The light intensity during the 12-hour day period was 3800 fc and no light was provided at night.

The purpose of the first experiment was to determine the concentration of diuron that would cause a 50% reduction in the growth of the diuron-susceptible variety, H 53-263.

Eighteen plants were established in nutrient solution and were placed in a growth chamber. After 1 week the plants were divided into 6 groups of 3 plants each, and since the plants were of different sizes, distribution within groups was based on the initial weights. The following diuron treatments were applied: 0, 0.1, 0.3, 0.4, 0.5, and 0.7 ppm. Plants were weighed initially and at 8 days after treatment; the gain in weight for each plant was determined and an I_{50} value was calculated.

The second experiment, designed to determine an I_{50} value for variety H50-7209, was similar to the first except that the concentration of diuron necessary for 50% growth inhibition of the diuron resistant variety, H 50-7209, was known from preliminary studies to be considerably higher. The diuron concentrations applied were as follows: 0, 1, 2, 3, 4, and 5 ppm.

The purpose of the third experiment was to determine the concentration of the primary diuron metabolite, 1-(3,4-dichlorophenyl)-3-methylurea, which would cause a 50% reduction in the growth of the susceptible variety, H 53-263. Fifteen plants of variety H 53-263 were established in 1 liter of nutrient solution placed in a growth chamber set for the same conditions as the previous experiments. The

plants were divided into 5 groups of 3 plants each, again distributed within groups on the basis of size. Monomethyl-diuron concentrations of 0, 0.5, 0.7, 0.9 and 1.2 ppm were added to nutrient solutions.

Sand Culture Studies. Single-eye seed pieces of 4 sugarcane varieties were established in a sub-irrigated sand culture system located on the Manoa Campus of the University of Hawaii. The experiment was a randomized complete block with a factorial arrangement of treatments (4 varieties x 2 levels of herbicide). The varieties screened for sensitivity to diuron were H 50-7209, H 57-5174, H 44-3098, and H 53-263; the treatments were diuron at 3 ppm and an untreated control. Each sand culture contained 4 plants and each treatment was replicated 3 times. At 41 days the heights of the plants were measured from the surface of the sand to the tip of the longest leaf; tillers were counted and dry weights taken.

In another experiment single-eye seed pieces of 3 sugarcane varieties, H 50-7209, H 57-5174, and H 53-263, were established as in the first experiment and were treated with 0, 1, and 3 ppm of diuron. The heights of the plants were measured 33 and 43 days after treatment, but instead of measuring the height of the plants to the tip of the

longest leaf, measurement was made from the base of the plant to the first visible dewlap. The experiment was a randomized complete block with a 3 x 3 factorial arrangement of treatments; there were 3 replications of 2 plants per treatment.

Uptake and Distribution Studies

Uptake. An experiment was designed to determine if uptake and/or distribution of carbonyl-labeled diuron was sufficiently different in the resistant variety, H 50-7209, and the susceptible variety, H 53-263, to explain the observed differential tolerance. Three plants of approximately equal size of varieties H 53-263 and H 50-7209 were placed in separate containers and each was treated with 22.7×10^5 cpm of carbonyl-labeled diuron. The plants were grown in aerated nutrient solution at a constant temperature of 85°C and relative humidity of 70%. The light and dark periods were of 12-hour duration. The experiment was initiated during the light period.

To determine the amount of diuron removed from the nutrient solution by the 2 varieties, 0.2 ml aliquots were removed periodically over a 228-hour period. The aliquots were placed directly into scintillation vials and taken to dryness in a 75°C oven. The vials were filled with 15 ml

of scintillation solution containing 0.5 g/l dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene] and 5 g/l PPO (2,5-diphenyloxazole) in toluene and were counted in a Beckman Liquid Scintillation System No. 150.

Distribution. The plants utilized in the uptake study were harvested at 14 days after treatment, dried and divided into the following parts: roots, old leaves (leaves 4-8), young leaves (leaves younger than number 4), old stem and young stem. The plant parts were dry homogenized in a Sorvall Omnimixer, then oven dried at 75°C for 48 hours. Subsequently, 20 mg samples were placed in a scintillation vial. Scintillation solution was added and the samples were assayed in the wide window (C^{14} + H^{3}). The distribution data were expressed as dpm/mg dry weight and on a total activity basis.

Metabolism Studies

An experiment was designed to determine if diuron was differentially degraded in resistant and susceptible varieties of sugarcane. Sugarcane plants of the susceptible variety, H 53-263, and the resistant variety, H 50-7209, were placed in nutrient solution. Three plants of each variety were transferred 1 week later to each of 4 one-gallon porcelain containers filled with 3 liters of nutrient solution and

0.3 ml of carbonyl-labeled ^{14}C diuron stock solution containing 5 uc/ml. One container was established as a fortified check with no sugarcane. The containers were placed in a growth chamber set for the following conditions: day temperature 85°F; night temperature 75°F; day humidity 60%; night humidity 90%. The duration of the day and night periods was 12 hours and the light intensity was maintained at 3800 fc.

At 1 and 3 weeks after treatment, 1 plant was removed from each of the 8 containers (4 replicates of each variety). Each plant was separated into roots, stems, and leaves. The separated plant parts were frozen and then extracted for diuron and other acetone-soluble residues. The ^{14}C diuron-fortified nutrient solution check was assayed at 1 week after treatment.

Extraction Procedure. The root, stem, and leaf tissue of the 2 sugarcane varieties were extracted in acetone. The first replication of the one-week samples was extracted from fresh-frozen material; all other extractions were made from samples which were frozen then dried in a 75°C oven for 48 hours. The fresh samples were extracted in 400 ml of acetone and the dried samples in 150 ml of acetone. Both extractions were made in a Sorvall Omnimixer. The extracts were filtered

and the filtrate was taken to dryness in an 80°C water bath (Method 2), or in a flash evaporator (Method 1). The extracts were redissolved in 20 ml of acetone which was later evaporated to 1 ml or less. The diuron-fortified nutrient solution was taken to dryness in a flash evaporator. The residue was redissolved in acetone and evaporated to approximately 1 ml.

Chromatography (Method 1). Eastman precoated silica gel thin-layer sheets (type 301R) were satisfactory for separation of diuron from its monomethyl and demethylated derivatives. The Eastman sheets have a fluorescent indicator which was used to determine the Rf values for unlabeled standards of diuron, monomethyldiuron, 1-(3,4-dichlorophenyl)-3-methylurea, and demethylated diuron, 3,4-dichlorophenylurea. A mix of the above standards was prepared and spotted at the 2 sides of the thin-layer sheet and under each of the spots of the plant extracts. A solvent system composed of benzene:acetone (2:1 v/v) was used for developing the pre-coated thin-layer sheets. The non-radioactive standards were visualized under short-wave ultraviolet light. The spots were circled in pencil, removed and identified as the origin, demethylated diuron, monomethyldiuron and diuron. The spots were placed in scintillation vials which were

filled with 15 ml of scintillation solution containing 0.5 g/l dimethyl POPOP and 5 g/l PPO in toluene. The samples were counted in the Beckman Scintillation System 150 with the use of the wide $^{14}C + {}^{3}H$ window.

Chromatography (Method 2). In the second method, Baker-flex silica gel S pre-coated strips were substituted for Eastman thin-layer sheets, primarily because a larger portion of extract could be spotted. A sugarcane root extract known to contain monomethyldiuron, demethylated diuron, and diuron was used as a standard for determination of Rf values. Benzene:acetone (2:1 v/v) was used as the solvent system. In order to place a maximum amount of radioactivity on the strips, 4 or 5 spots were placed at the origin. After developing, the strips were cut into 1 cm sections beginning 0.5 cm below the origin and continuing to the solvent front. The sections were placed in scintillation vials which were filled with 15 ml of scintillation solution and were assayed as described above.

RESULTS AND DISCUSSION

Phytotoxicity Studies

Field experiment. The effect of diuron and ametryne on the growth in height of varieties H 50-7209, H 57-5174, and H 53-263 is summarized in Table I. (Additional data is given in Appendix Tables I-V). The growth of the 3 varieties was found to differ significantly at all dates of measurement; however, the herbicide treatments were significantly different only at 46 days. The interaction between variety and herbicides was significant at all dates of measurement which places only minor importance on the variety and treatment main effects. The significant interaction between variety and treatment indicated that the varieties were differentially susceptible to diuron and ametryne. Variety H 53-263 was found to be susceptible to both diuron and ametryne while varieties H 57-5174 and H 50-7209 were found to be tolerant. Orthogonal comparisons of the sugarcane height data are given in Table II.

The variety main effect was subdivided into 2 orthogonal comparisons: H 53-263 vs. H 57-5174, and H 50-7209 and H 57-5174 vs. H 50-7209; both were significant at all dates of measurement indicating that H 53-263 was significantly shorter than H 57-5174 and H 50-7209, and that H 57-5174 was

TABLE I. EFFECT OF DIURON AND AMETRYNE ON THE GROWTH OF THREE SUGARCANE VARIETIES AT FIVE DATES OF MEASUREMENT
(See Appendix Tables I-V)

Variety	Treatment (lb./acre)	Height to First Visible Dewlap (cm.) Days after Treatment 46	58	71	92	138
H 50-7209	Check	23.9 1/	28.3	40.3	66.8	127.4
	Diuron 9	28.3	37.4	48.6	78.2	144.0
	Ametryne 9	28.8	36.6	50.3	80.7	144.4
H 57-5174	Check	29.2	36.6	51.7	86.9	151.0
	Diuron 9	31.6	38.6	53.9	86.3	151.8
	Ametryne 9	30.0	36.0	52.2	89.2	159.9
H 53-263	Check	22.4	27.6	37.4	63.1	128.1
	Diuron 9	22.5	25.2	34.5	53.6	102.6
	Ametryne 9	19.6	20.5	27.0	42.5	95.1
Significance						
Treatment mean square		**	NS	NS	NS	NS
Variety mean square		**	**	**	**	**
Interaction mean square		**	**	*	**	**
Coefficient of variation %		5.8	8.0	9.6	9.0	6.5

1/ Average of 36 values in three replications.

TABLE II. ORTHOGONAL COMPARISONS OF SUGARCANE HEIGHT DATA

Comparisons	Days after Treatment				
	46	58	71	92	138
Main Effects (mean squares)					
1. H 53-263 vs. H 57-5174 and H 50-7209	305.3**	744.4**	1640.1**	4778.9**	8557.9**
2. H 57-5174 vs. H 50-7209	48.7**	40.2*	174.2**	674.7**	1095.1**
3. Check vs. diuron and ametryne	16.0*	13.9	10.2	1.6	39.5
4. Diuron vs. ametryne	8.0*	32.5*	28.4	16.4	0.3
Interactions (mean squares)					
1 x 3	26.1*	119.3**	187.2**	634.1**	2128.0**
1 x 4	5.1	9.7	55.3	190.0	134.6
2 x 3	9.8*	64.3**	61.9	138.8	146.4
2 x 4	3.3	2.3	8.3	0.1	0.5
Error mean square	1.8	6.6	15.6	42.6	76.37
Coefficient of variation %	5.8	8.0	9.6	9.0	6.5

significantly taller than H 50-7209. The herbicide main effects were also divided into orthogonal comparisons: check vs. diuron and ametryne; diuron vs. ametryne. The former was significant only at 46 days; the latter was significant at 46 and 58 days. The former comparison indicates that after 46 days the diuron and ametryne-treated sugarcane was equal to the check when all the varieties were considered; the latter comparison indicates that there were no differences between diuron and ametryne treatments after 58 days. Up to 58 days ametryne was more toxic to H 53-263 than diuron. Neither H 50-7209 nor H 57-5174 was susceptible to either the diuron or the ametryne treatments at any date of measurement; in fact, with both varieties the diuron and ametryne-treated plants were taller than the checks up to 58 days after treatment (2 x 3 interaction, Table II).

When the diameter of the cane stalks was measured at 138 days, the variety and treatment main effects, as well as interaction, were also found to be significant (Table III). For additional data see Appendix Table VI. The main effects and interactions were orthogonally divided (Table IV). Variety H 57-5174 had smaller diameter stalks than H 50-7209, and the diuron-treated plants had stalks of smaller diameter than the ametryne-treated plants when all the varieties were

considered. There was no effect of either herbicide on variety H 50-7209, but reductions in stalk diameter were noted for plants of H 57-5174 and H 53-263 which were treated with diuron. Ametryne-treated plants of H 53-263 had stalk diameters which were smaller than the controls. On the other hand, ametryne-treated plants of H 57-5174 and H 50-7209 had larger diameters than the controls. The variety x treatment interaction was significant, indicating that the varieties were differentially susceptible to the diuron and ametryne. The coefficient of variation for the diameter data was only 3 percent at 138 days.

When the tillering data were taken at 188 days after treatment, the variety and treatment main effects were significant as well as the interaction between variety and treatment (Table III). For additional data see Appendix Table VII. Variety H 57-5174 produced a greater number of tillers than either H 50-7209 or H 53-263. Tillering was reduced by both diuron and ametryne in all varieties, but the difference between the controls and the treated plants was greatest in the diuron-treated plants of H 57-5174 and the diuron- and ametryne-treated plants of H 53-263. The coefficient of variation for the tillering data was 8.4%.

TABLE III. EFFECT OF DIURON AND AMETRYNE ON THE GROWTH IN DIAMETER AND TILLERING OF THREE SUGARCANE VARIETIES
(See Appendix Tables VI and VII)

Variety	Treatment (lb./acre)	Diameter (cm) (138 days)	Number of stalks/stool (188 days)
H 50-7209	Control	2.88	8.4
	Diuron 9	2.95	7.9
	Ametryne 9	2.94	7.6
H 57-5174	Control	2.68	12.2
	Diuron 9	2.56	10.6
	Ametryne 9	2.79	11.2
H 53-263	Control	3.02	10.5
	Diuron 9	2.63	6.8
	Ametryne 9	2.78	8.0
Significance			
Treatment mean square		*	**
Variety mean square		**	**
V x T mean square		*	*
Coefficient of variation %		3.0	8.4

TABLE IV. ORTHOGONAL COMPARISONS OF SUGARCANE DIAMETER AND TILLERING DATA (Values are Mean Squares).

Comparisons	Diameter (138 days)	Tillering (188 days)
Main Effects		
1. H 53-263 vs. H 57-5174 and H 50-7209	0.000	6.54**
2. H 57-5174 vs. H 50-7209	0.276**	41.71**
3. Control vs. diuron and ametryne	0.045	11.03**
4. Diuron vs. ametryne	0.072*	0.98
Interactions		
1 x 3	0.172**	9.66**
1 x 4	0.003	1.10
2 x 3	0.006	0.10
2 x 4	0.040	0.52
Mean square error	0.10	0.60
Coefficient of variation	3.0%	8.4%

Nutrient Solution Experiments. Data showing the effect of diuron on the growth of the susceptible variety, H 53-263, in nutrient solution are given in Table V. Additional data is given in Appendix Table VIII. The concentration of diuron necessary to bring about a 50% reduction in the fresh weight of variety H 53-263 I_{50} was 0.35 ppm. A large portion of the growth inhibition in the diuron-treated plants was due to a rather severe inhibition of root growth. Whether the root inhibition is a primary effect of diuron on the root tissue or whether it is a secondary symptom of the diuron-induced inhibition of photosynthesis is not known. Typical leaf chlorosis on the older leaves, which is often reported as a diuron symptom in sugarcane, was absent. Evidence of phytotoxicity appeared as inhibition of both top and root growth.

Data showing the effect of diuron on the resistant variety, H 50-7209, are given in Table VI. The I_{50} value for diuron on variety H 50-7209 was determined to be 1.2 ppm, or 4.5 times greater than the value for the susceptible variety, H 53-263. The symptoms of diuron injury for varieties H 50-7209 and H 53-263 were manifested in inhibition of top and root growth rather than chlorosis.

The primary diuron metabolite, monomethyldiuron, was

found to be 2.5 times less toxic to variety H 53-263 than diuron (Table VII). The I_{50} value for monomethyl diuron on variety H 53-263 was determined to be 0.9 ppm. Raw data for the nutrient solution experiments are found in Appendix Tables VIII, IX, and X.

Sand Culture Experiment. Data showing the effect of diuron on the growth of 4 varieties of sugarcane 41 days after treatment is given in Table VIII. The height data in this preliminary experiment were extremely variable, and treatment, variety, and variety x treatment interaction were not significant. However, when the dry weight data were analyzed, variety and treatment were significant, but there was no interaction between the two. In addition to decreasing the dry weight, diuron severely inhibited the tillering of all varieties. The symptoms of diuron injury differed depending upon variety. At 41 days after treatment, variety H 53-263 was either dead or dying, variety H 57-5174 was severely inhibited and varieties H 50-7209 and H 44-3098 were relatively unaffected.

Table IX shows the effect of diuron on the growth in height of the three varieties of sugarcane when measured 33 and 43 days after treatment. The height data were more uniform than in the first experiment; therefore differences

TABLE V. EFFECT OF DIURON ON VARIETY H 53-263 IN NUTRIENT SOLUTION
(See Appendix Table VIII)

Treatment (ppm diuron)	Average Gain in Fresh Weight (over 8-day period) (grams)	% Inhibition
0.0 (check)	7.26 1/	0.0
0.1	5.43	25.3
0.3	4.20	42.2
0.4	3.23	55.6
0.5	2.60	64.2
0.7	1.06	85.7

TABLE VI. EFFECT OF DIURON ON VARIETY H 50-7209 IN NUTRIENT SOLUTION
(See Appendix Table IX)

Treatment (ppm diuron)	Average Gain in Fresh Weight (over 8-day period) (grams)	% Inhibition
0.0	18.0 1/	0.0
1.0	10.5	42.6
2.0	6.8	62.2
3.0	7.3	59.9
4.0	7.2	60.0
5.0	4.9	72.8

1/ Average of three replications.

TABLE VII. EFFECT OF MONOMETHYLDIURON ON VARIETY H 53-263 IN NUTRIENT SOLUTION
(See Appendix Table X)

Treatment (ppm diuron)	Average Gain in Fresh Weight (over 8-day period) (grams) 1/	% Inhibition
0.0	11.2	0.0
0.5	8.7	23.5
0.7	6.5	42.0
0.9	5.6	50.0
1.2	2.5	78.1

1/ Average of three replications.

TABLE VIII. EFFECT OF DIURON ON THE GROWTH OF FOUR VARIETIES OF SUGARCANE IN SAND CULTURE
(See Appendix Tables XI-XIII)

Variety	Treatment	Height of longest leaf (cm)	Dry Wt. of tops (gr.)	Tillering (No.)
H 50-7209	Control	117.4 ab 1/	20.68 ab	3.3
	Diuron 3 ppm	145.8 a	17.19 bcd	1.3
H 44-3094	Control	107.6 b	11.42 bcde	5.0
	Diuron 3 ppm	91.6 b	5.48 efg	1.0
H 57-5174	Control	111.3 ab	17.38 bc	7.0
	Diuron 3 ppm	89.8 b	5.00 efg	0.0
H 53-263	Control	102.6 b	29.00 a	7.3
	Diuron 3 ppm	78.6 b	6.05 ef	0.0

1/ Each value is an average of four replications; means followed by same letter are not significantly different at 5% level (Duncan's Multiple Range Test).

TABLE IX. EFFECT OF DIURON ON THE GROWTH OF THREE VARIETIES OF SUGARCANE AT 33 AND 43 DAYS AFTER TREATMENT (See Appendix Tables XIV and XV)

Variety	Treatment	Height to first visible dewlap (cm) 33 Days	43 Days
H 50-7209	Control	23.5 a[1/]	29.1 a
	Diuron 1 ppm	21.5 a	29.1 a
	Diuron 3 ppm	20.2 abc	26.3 ab
H 57-5174	Control	17.6 cde	21.1 c
	Diuron 1 ppm	12.16 fgh	15.7 d
	Diuron 3 ppm	9.0 gh	9.6 f
H 53-263	Control	20.0 abcd	22.8 bc
	Diuron 1 ppm	15.2 ef	15.1 de
	Diuron 3 ppm	12.7 fg	12.7 df

1/ Each value is an average of three replications; means followed by same letter are not significantly different at 5% level (Duncan's Multiple Range Test).

between treated and untreated plants are more apparent. Varieties H 57-5174 and H 53-263 were more severely affected by diuron than variety H 50-7209. Treated plants of H 53-263 were dead or dying at the termination of the experiment, while plants of variety H 57-5174 were inhibited severely in growth. There was a significant interaction between varieties and treatments, indicating that there were differences in the tolerance to diuron between the varieties tested.

The sand culture experiments showed that sugarcane varieties were differentially tolerant of diuron. The order of increasing tolerance, based on growth in height of the sugarcane plant, was as follows: H 53-263, H 57-5174, H 44-3098, and H 50-7209. This data is in agreement with that of Hilton and Nomura (1966) except for variety H 57-5174 which they classified as resistant to diuron (when measured through root absorption). The author would have to classify H 57-5174 as susceptible to diuron based on his data: cf. field experiment. Variety H 53-263 was dead or dying at the termination of both experiments, variety H 57-5174 was severely inhibited, and variety H 50-7209 was only slightly affected. A reduction in tillering was noted for all varieties treated with diuron. The tolerance of diuron as

exhibited by sugarcane varieties appears to be a characteristic of the variety. It should be noted that the conditions of the experiments reported herein were harsh, i.e. the cane roots could not outgrow the diuron since it was applied through a sub-irrigation system. Measurement of height to the first visible dewlap was more satisfactory than using height to the tip of the longest leaf. Raw data for the sand culture studies are given in Appendix Tables XI-XV.

The field, sand culture, and nutrient solution experiments showed that sugarcane varieties were differentially susceptible to diuron. In the field variety H 53-263 was susceptible to both ametryne and diuron, while varieties H 50-7209 and H 57-5174 were resistant. The height, diameter and tillering measurements were sensitive enough to detect small differences in growth without extensive replications and are recommended for use in future yield studies with herbicides in Hawaiian sugarcane. The critical experiment which should be conducted in Hawaii is one in which early herbicide-induced growth reductions in susceptible varieties are correlated with two-year yield of sugar. If a correlation cannot be made then growers could further mechanize their weed control operations with considerable savings. Some recent unpublished data in a highly replicated two-year yield

test showed that the yield of sugar at 2 years was substantially increased in the susceptible variety H 53-263 which was severely injured by diuron (unpublished data, H.S.P.A.). This increase may have occurred because stalk populations were reduced, thus decreasing the self-competition between stools. There was enough recovery during the second year of growth to overcome the early damage. In the same test, ametryne was not as toxic to H 53-263 and the yield was not significantly different from the check.

In sand culture varieties H 53-263 and H 57-5174 were found to be susceptible to diuron, while variety H 50-7209 was found as tolerant. The effect of diuron on the tillering which was observed in the field was even more evident in the sand culture studies. Both varieties H 57-5174 and H 53-263 produced no tillers when treated with diuron at 3 ppm.

In the nutrient solution studies, diuron was found to be 4.5 times more toxic to H 53-263 than to H 50-7209, and the primary diuron metabolite, monomethyldiuron, was more toxic to H 53-263 than diuron was to H 50-7209. Symptoms of diuron injury observed in nutrient solution and sand culture appeared as reduced growth and poor tillering, while in the field the reduced growth was associated with chlorosis. The differences between the field observations and the pot

experiments are probably due to differences in nutrition or light intensity. When nutrition is adequate and light intensity is low, symptoms of injury tend to appear as growth inhibition rather than chlorosis. Generally, plants which are under stress of any kind are most susceptible to diuron injury.

Uptake and Distribution Studies

Uptake. Table X shows the amount of diuron (radioactivity) absorbed or adsorbed from nutrient solution by the resistant variety, H 50-7209, and the susceptible variety, H 53-263. The table also indicates the percentage of the original treatment solution removed from the nutrient solution over the 228-hour time period. At 1/4 hour both varieties removed approximately 15% of the radioactivity, but from 6 to 37 hours, variety H 53-263 removed about 10% more radioactivity than H 50-7209. From 120 through 228 hours the uptake was approximately equal. At the termination of the experiment (228 hours after treatment) the amount of radioactivity removed from the nutrient solution by both varieties was essentially the same.

Distribution. Table XI shows the distribution of radioactivity expressed as dpm/mg dry weight in 2 varieties of sugarcane 14 days after treatment with carbonyl-labeled

diuron, and Table XII shows the distribution of radioactivity in the same plants in terms of percent of total radioactivity. The distribution data calculated on a dry weight basis show that the concentration of radioactivity in the roots of H 50-7209 was twice that of H 53-263, and that the concentration in the leaves younger than No. 4 was twice as great in H 53-263 compared to H 50-7209. The concentration in the older leaves and the stems of the 2 varieties was almost equivalent. Table XIII gives the recovery data for the radioactivity applied to the nutrient solution in which the 2 varieties were growing. Recoveries were low; they ranged from 16.8 to 6.8% with the method employed. Low recovery may be due to some of the following: breakage of carbonyl bonds and subsequent release of CO_2 or other volatile substances, adsorption to the sides of plastic containers, deposition in cotton plugs at top of the container due to breaking of aeration bubbles, loss in wash water, loss while grinding samples, volatility loss during sample drying and self-absorption of soft beta rays within fibrous material in scintillation vials. All of these potential sources of radioactivity loss should be considered in future work.

The uptake of carbonyl-labeled diuron from nutrient

solution was essentially the same for both the resistant and susceptible varieties; however, distribution of the carbonyl group within the plant in terms of dpm/mg was considerably different. The resistant H 50-7209 variety contained about 2 times as much activity in the roots as the susceptible variety, H 53-263. Conversely, there was about 2 times as much activity in the young leaves of variety H 53-263 compared to H 50-7209. Since the concentration of radioactivity in the resistant variety was greater in the roots than in the young leaves, with the converse being true in the susceptible variety, it is proposed that differential distribution was at least partially responsible for the observed differences in varietal tolerance.

Metabolism Studies

Since 2 extraction and chromatography procedures were used, the results of the metabolism studies will be given in 2 sections. In the first section the data from the first replication of the one-week samples are presented, and in the second section data are presented for the remaining samples.

Section 1. Data showing the percent of radioactivity present as diuron and diuron metabolites at 1 week after treatment are given in Table XIV. Values for a diuron

TABLE X. UPTAKE OF DIURON FROM NUTRIENT SOLUTION BY VARIETIES H 53-263 AND H 50-7209 NINE DAYS AFTER TREATMENT WITH CARBONYL-LABELED DIURON

Hours after Treatment	Diuron Removed (cpm x 10^5)		Percent Removed 1/	
	H 50-7209	H 53-263	H 50-7209	H 53-263
1/4	4.2	3.6	15.4	13.2
6	7.2	10.1	26.5	37.1
15	8.2	11.5	30.1	42.3
37	11.7	15.1	43.0	55.5
120	16.3	18.7	59.9	60.9
168	19.3	20.0	71.0	73.5
228	22.6	22.4	83.0	82.5

1/ Each plant received 27.2 x 10^5 cpm. Values are an average of three replications.

TABLE XI. DISTRIBUTION OF RADIOACTIVITY IN TWO SUGARCANE VARIETIES 14 DAYS AFTER TREATMENT WITH CARBONYL-LABELED DIURON (PERCENT OF TOTAL RADIOACTIVITY)[1/]

Plant Part	Variety	
	H 50-7209	H 53-263
Roots	31.6	29.0
Leaves 4-9[2/]	45.2	48.4
Leaves younger than 4	7.1	8.3
Stem (old)	13.1	12.7
Stem (young)	2.7	1.5

TABLE XII. DISTRIBUTION OF RADIOACTIVITY PER UNIT OF DRY WEIGHT IN TWO SUGARCANE VARIETIES 14 DAYS AFTER TREATMENT WITH CARBONYL-LABELED DIURON (DPM/mg)[1/]

Plant Part	Variety	
	H 50-7209	H 53-263
Roots	45.3	21.4
Leaves 4-9[2/]	33.4	34.8
Leaves younger than 4	15.3	30.9
Stem (old)	21.1	14.5
Stem (young)	20.7	13.8

1/ Values are an average of 3 replications.

2/ The spindle leaf is designated No. 1; older leaves are given consecutively higher numbers.

TABLE XIII. RECOVERY OF RADIOACTIVITY FROM SUGARCANE PLANTS TREATED WITH 2.91×10^6 DPM OF CARBONYL-LABELED DIURON 14 DAYS AFTER APPLICATION TO NUTRIENT SOLUTION

	Replication		
	1	2	3
Variety H 50-7209			
Amt. absorbed or lost (DPM)	2.68×10^6	2.12×10^6	2.40×10^6
Amt. recovered in plant (DPM)	$.45 \times 10^6$	$.28 \times 10^6$	$.21 \times 10^6$
Percent Recovery	16.8	13.2	8.7
Variety H 53-263			
Amt. absorbed or lost (DPM)	2.35×10^6	2.40×10^6	2.38×10^6
Amt. recovered in plant (DPM)	$.16 \times 10^6$	$.21 \times 10^6$	$.22 \times 10^6$
Percent Recovery	6.8	8.7	9.2

standard and a sample of fortified nutrient solution are given in the same table for comparison. The data show that diuron was metabolized to monomethyldiuron and demethylated diuron in the roots, stems, and leaves of both the resistant variety, H 50-7209 and the susceptible variety, H 53-263. In Table XV these data are further summarized to indicate the percentage of toxic residue (diuron and monomethyldiuron) and other residues (demethylated diuron and undesignated radioactivity). The roots of the 2 varieties contained approximately equal amounts of toxic and non-toxic compounds; however, the stems and leaves contained a considerably larger portion of the radioactivity as toxic residue in the susceptible variety.

Section 2. Data showing the breakdown of diuron in the remaining 1-week and 2-week samples are given in Tables XVI and XVII, respectively. As shown in the earlier data, both varieties metabolized diuron. The Rf values for diuron, monomethyldiuron, and demethylated diuron using Baker-flex pre-coated Silica gel S TLC sheets were .54, .43, and .29, respectively. Using method I, the total activity on each TLC strip was assayed only where specific spots were removed corresponding to Rf values for diuron, monomethyldiuron, demethylated diuron, and the origin. The second method made

it possible to assay the radioactivity occurring in the tails of the peaks; however, the method was less sensitive for determining the identity of the parent diuron and its metabolites. Based on the chromatography and strip counting of a standard solution known to contain radioactive diuron, monomethyldiuron, and demethylated diuron, it was determined that the radioactivity between Rf .45 and .85 was due largely to diuron, while the activity between .05 and .45 was due primarily to the presence of monomethyldiuron and demethylated diuron. On several TLC strips considerable activity occurred at the origin or at the solvent front. The activity occurring in these locations was termed "undesignated activity."

The distribution of radioactivity on TLC strips spotted with extracts from roots, stems, and leaves of variety H 50-7209 and H 53-263 at 1 and 3 weeks after treatment is shown in Appendix Tables XVI and XVII. The summarized data in Tables XVI and XVII shows that at 1 and 3 weeks after treatment there was a considerably greater amount of radioactivity present as diuron in the susceptible variety, H 53-263, when compared to the resistant variety, H 50-7209. The difference is greater in the leaves where the primary site of phenylurea activity is located. At 1 and 3 weeks

TABLE XIV. METABOLISM OF DIURON IN ROOTS, STEMS, AND LEAVES OF VARIETIES H 50-7209 AND H 53-263 AT ONE WEEK AFTER TREATMENT

	Residue (Percent)[1/]			
	Diuron	Monomethyldiuron	Demethylated diuron	Unknown
Roots				
H 53-263	33	47	18	2
H 50-7209	46	35	12	7
Stems				
H 53-263	35	45	15	5
H 50-7209	26	33	23	18
Leaves				
H 53-263	47	37	12	4
H 50-7209	24	25	19	32
Diuron Std.	95.0	2.5	1.0	1.5
Fortified nutrient solution	92.2	4.6	1.8	1.6

1/ Values are the percent of radioactive residue at the Rf values corresponding to diuron, monomethyldiuron, demethylated diuron and an unknown compound at Rf 0.

TABLE XV. PRODUCTION OF TOXIC AND OTHER RESIDUE BY TWO VARIETIES OF SUGARCANE (ONE WEEK)

	Residue (Percent)	
	Toxic Residues[1/]	Other Residues[2/]
Roots		
H 53-263	80	20
H 50-7209	81	19
Stems		
H 53-263	80	20
H 50-7209	59	41
Leaves		
H 53-263	84	16
H 50-7209	49	51

1/ Includes diuron and monomethyldiuron.

2/ Includes demethylated diuron and unknown at Rf = 0.

TABLE XVI. METABOLISM OF DIURON BY ROOTS, STEMS, AND LEAVES OF H 50-7209 AND H 53-263 (ONE WEEK)
(See Appendix Table XVI)

	Percent of Total Radioactivity[1]		
	Diuron	Known Metabolites[2]	Undesignated Activity[3]
Roots			
H 50-7209	35.2	57.1	7.5
H 53-263	59.1	28.2	12.1
Stems			
H 50-7209	26.5	53.2	20.3
H 53-263	40.9	31.8	27.3
Leaves			
H 50-7209	16.5	64.4	18.5
H 53-263	67.0	24.8	8.3
Fortified Sample	79.1	12.7	8.0
Diuron Std.	80.6	8.7	10.4

1/ Average of three replications of each variety.

2/ Monomethyldiuron and demethylated diuron.

3/ Activity at origin and solvent front.

TABLE XVII. METABOLISM OF DIURON BY ROOTS, STEMS, AND LEAVES OF H 50-7209 AND H 53-263 (3 WEEKS)
(See Appendix Table XVII)

	Percent of Total Radioactivity[1]		
	Diuron	Known Metabolites[2]	Undesignated Activity[3]
Roots			
H 50-7209	35.0	50.7	14.2
H 53-263	49.9	40.4	9.4
Stems			
H 50-7209	22.7	33.1	22.1
H 53-263	55.0	38.6	6.4
Leaves			
H 50-7209	22.4	58.4	19.0
H 53-263	63.8	31.8	4.3

1/ Average of 4 replications (H 50-7209)--
3 replications (H 53-263)

2/ Monomethyldiuron and demethylated diuron.

3/ Activity at origin and solvent front.

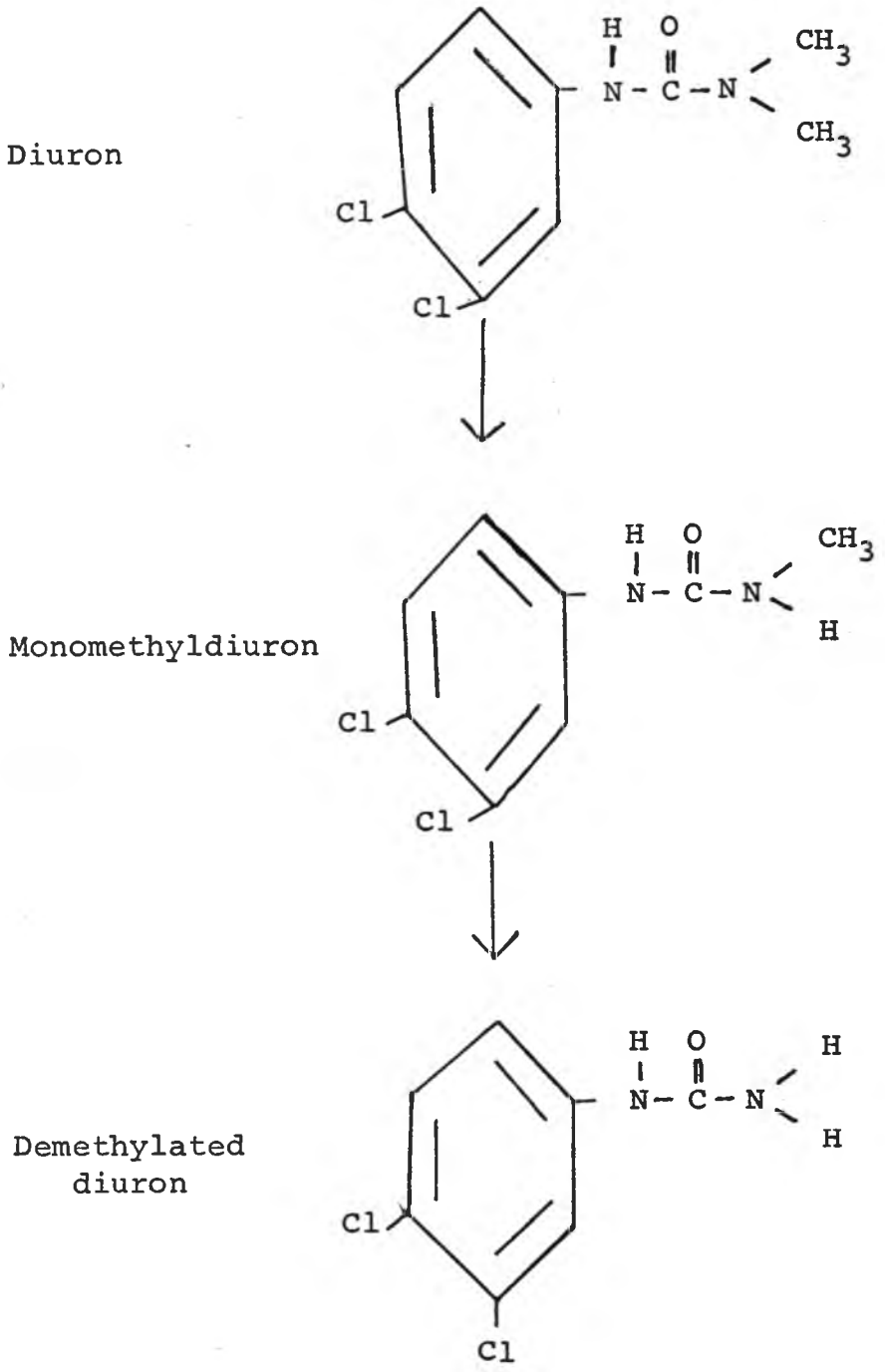

Figure 1. Degradation of carbonyl-labeled diuron by sugarcane.

after treatment there was approximately 3 times as much radioactivity present as diuron in the leaves of H 53-263 when compared to H 50-7209. There was not a great deal of difference between the 1 and 3 week samples, indicating that metabolism occurs rapidly and then levels off.

Figure 1 shows the proposed pathway for the degradation of carbonyl-labeled diuron in varieties H 53-263 and H 50-7209. One should keep in mind that although the same metabolites were formed in both varieties, the metabolites occurred in higher yield in the resistant variety.

Both differential distribution and degradation of diuron appear to contribute to the relative toxicity of diuron to varieties H 53-263 and H 50-7209. Data in Table XII show that the diuron concentration in the young leaves was approximately 2 times greater in variety H 53-263 compared with H 50-7209. Distribution in the older leaves was approximately equal. By averaging the data for young and old leaves there was 1.3 times more radioactive residue in the leaves of H 53-263 compared with H 50-7209. This would include diuron, monomethyldiuron and demethylated diuron. In contrast to the distribution in leaves, variety H 50-7209 contained 2 times more radioactive residue in the roots compared with H 53-263.

Table XVII shows that diuron was more completely degraded in variety H 50-7209. Diuron remaining in the leaves of variety H 50-7209 was only 22% of the total radioactive residue, while for variety H 53-263 the diuron constituted 63.8% of the residue. Combining the information from the distribution and metabolism studies, one can account for approximately a 4-fold difference in the concentration of diuron in the leaves of resistant and susceptible varieties. Phytotoxicity data from nutrient solution indicated that variety H 50-7209 was 4.5 times more tolerant of diuron compared with variety H 53-263 (Tables V and VI).

Although diuron and ametryne toxicity to variety H 53-263 was demonstrated up to 4.5 months in the field, it should be noted that sugarcane is grown in Hawaii on a 2 to 3 year crop cycle, and that injury which occurs early in the crop cycle may have no relation to final harvest. There is no evidence that herbicides used at adequate rates for weed control, even on susceptible varieties, are reducing the yield of two-year sugarcane in Hawaii.

SUMMARY

Hawaiian sugarcane varieties were found to be differentially susceptible to both diuron and ametryne in a field experiment. Variety H 53-263 was susceptible while varieties H 57-5174 and H 50-7209 were found to be tolerant. These findings were in agreement with results obtained on Hawaiian plantations except for variety H 57-5174 which has been notably susceptible to post-emergence applications of herbicides. Since post-emergence applications in the author's experiment were made as directed sprays with care taken not to excessively cover the foliage, variety H 57-5174 may have escaped injury.

In nutrient solution variety H 53-263 was found to be 4.5 times more susceptible to root-applied diuron compared to H 50-7209. The concentrations of diuron necessary for a 50% reduction in the fresh weight of varieties H 50-7209 and H 53-263 were 1.6 ppm and 0.35 ppm, respectively.

Sand culture studies of diuron phytotoxicity to sugarcane varieties resulted in severe injury to H 53-263, moderate injury to H 57-5174, and only minor injury to H 50-7209. Reduction in tillering as a result of diuron applications was especially evident in sand culture.

The uptake, distribution and metabolism of diuron by

a resistant and a susceptible variety of sugarcane were studied as possible causes of the observed differential tolerance. Both the resistant and susceptible varieties removed approximately 80% of root-applied carbonyl-labeled diuron from nutrient solution at 9 days after treatment. Therefore differential absorption was not considered to be a contributing factor to the differential tolerance.

The distribution of radioactivity in the resistant and susceptible varieties at 14 days after treatment was almost identical when calculated on a total activity basis; however, when calculated on the basis of activity per unit of dry weight, there was a greater concentration of diuron and metabolites in the younger leaves of the susceptible variety compared to the resistant variety. Conversely, there was more diuron and metabolites in the roots of H 50-7209 than in H 53-263. Since the site of activity of diuron is in the leaves and a greater concentration of diuron was found in the young leaves of variety H 53-263, differential distribution of diuron was concluded to be at least partially accountable for differences in varietal tolerance.

Diuron was found to be degraded by successive demethylation in both the resistant and susceptible sugarcane varieties. The primary metabolites were monomethyldiuron

[1-(3,4-dichlorophenyl)-3-methylurea] and demethylated diuron, (3,4-dichlorophenylurea). The degradation of diuron was found to be more extensive, especially in the leaf and stem tissue of the resistant variety.

Since less diuron entered the leaves of variety H 50-7209 and since the diuron which entered the leaves was more thoroughly metabolized, it was proposed that the differential distribution and metabolism contribute to the relative toxicity of diuron to the varieties studied.

REFERENCES

Dalton, R. L., A. W. Evens and R. C. Rhodes (1966). Disappearance of diuron from cotton field soils. Weeds 14:31-33.

Hilton, H. W. and N. Nomura (1966). Unpublished data, Hawaiian Sugar Planters' Association.

Hilton, H. W., N. Nomura, and G. Uyehara (1965). Unpublished data, Hawaiian Sugar Planters' Association.

Millhollon, Rex W. and Rouby J. Matherne (1968). Tolerance of sugarcane varieties to herbicides. Weed Science 16:300-303.

Minshall, W. H. (1954). Translocation, path, and place of action of 3-(4-chlorophenyl)-1,1-dimethylurea in bean and tomato. Canadian Journal of Botany. 32:795-798.

Muzik, T. J., H. J. Cruzado, and A. J. Loustalot (1954). Studies on the absorption, translocation and action of CMU. Botanical Gazette 166:65-73.

Nolla, J.A.B. (1950). Injury to sugarcane from 2,4-D. Int. Soc. Sugarcane Technologists Proceedings, 7:178-189.

Olney, John H., George Yip, and Mary H. Aldridge (1968). A metabolic study of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) applied to corn seedlings. Agricultural and Food Chemistry 16:426-433.

Rochecouste, E. (1962). Phytotoxic effects of herbicides. Mauritius Sugar Industry Research Institute, Annual Report, pp. 72-74.

Rochecouste, E. (1963). Varietal susceptibility to DCMU. Mauritius Sugar Industry Research Institute, Annual Report, p. 96.

Rochecouste, E. (1964). Investigations on the use of substituted uracils. Report of Mauritius Sugar Industry Research Institute, pp. 75-76.

Rochecouste, E. (1967). Weed control in sugarcane. Mauritius Sugar Industry Research Institute, Réduit, Mauritius, pp. 68-69.

Rogers, R. L. and H. H. Funderburk, Jr. (1967). Absorption, translocation, and metabolism of cotoran by cotton and cucumber. Abst. Paper presented at the Weed Science Society of America 1967 meeting, p. 43.

Smith, J. W. and T. J. Sheets (1967). Uptake, distribution and metabolism of monuron and diuron by several plants. J. of Ag. and Food Chem. 15:577-581.

Swanson, C. R. and H. R. Swanson (1968a). Metabolic fate of monuron and diuron in isolated leaf discs. Weed Science 16:137-143.

Swanson, C. R. and H. R. Swanson (1968b). Inhibition of degradation of monuron in cotton leaf tissue by carbamate insecticides. Weeds 16:481-484.

Sweetser, Philip B. (1962). Photoinactivation of monuron, 3-(p-chlorophenyl)-1,1-dimethylurea, by riboflavin-5-phosphate. Biochemica et Biophysica Acta. 66:78-85.

Van Overbeek, J. (1964). Survey of mechanisms of herbicide action. The Physiology and Biochemistry of Herbicides. Academic Press, New York, pp. 387-400.

Wiemer, Robert D. (1963). The effect of several herbicides on eight sugarcane varieties. M. S. Thesis, Agronomy Dept. University of Hawaii, 55 pages.

A P P E N D I X

APPENDIX TABLE I. EFFECT OF DIURON AND AMETRYNE ON THE GROWTH IN HEIGHT OF THREE SUGARCANE VARIETIES 46 DAYS AFTER TREATMENT

Variety	Treatment		Height to first visible dewlap (cm) Replication			Average
	Lb. a.i./acre[2/]		1	2	3	
H 50-7209	Control		26.4[1/]	22.4	22.9	23.9
	Diuron	9	29.5	26.7	28.8	28.3
	Ametryne	9	29.8	28.3	28.3	28.8
H 57-5174	Control		30.2	31.3	26.3	29.2
	Diuron	9	31.9	31.9	31.1	31.6
	Ametryne	9	29.3	30.6	30.1	30.0
H 53-263	Control		22.2	21.6	23.4	22.4
	Diuron	9	22.9	21.8	22.8	22.5
	Ametryne	9	20.8	18.1	20.1	19.6

Analysis of Variance

Source of variation	DF	MS
Replication	2	3.5
Variety	2	177.0**
Treatment	2	12.0**
V X T	4	11.0**
Error	16	1.8
Total	26	

C.V. = 5.8

1/ An average of 12 measurements.

2/ 4 Lb./acre pre-plus 5 lb./acre post-emergence.

APPENDIX TABLE II. EFFECT OF DIURON AND AMETRYNE ON THE GROWTH IN HEIGHT OF THREE SUGARCANE VARIETIES 58 DAYS AFTER TREATMENT

Variety	Treatment		Height to first visible dewlap (cm) Replication			Average
	Lb. a.i./acre[2/]		1	2	3	
H 50-7209	Control		32.6[1/]	24.5	27.9	28.3
	Diuron	9	36.3	39.5	36.4	37.4
	Ametryne	9	39.3	35.8	34.8	36.6
H 57-5174	Control		37.9	38.3	33.8	36.6
	Diuron	9	38.9	39.5	37.4	38.6
	Ametryne	9	34.9	36.5	36.8	36.0
H 53-263	Control		27.6	27.2	28.2	27.6
	Diuron	9	22.7	26.6	26.5	25.2
	Ametryne	9	20.9	16.4	24.2	20.5

Analysis of Variance

Source of variation	DF	MS
Replication	2	1.4
Variety	2	392.3**
Treatment	2	23.2
V X T	4	48.9**
Error	16	6.6
Total	26	

C.V. = 8.0

1/ Average of 12 measurements.

2/ 4 Lb./acre pre-plus 5 lb./acre post-emergence.

APPENDIX TABLE III. EFFECT OF DIURON AND AMETRYNE ON THE GROWTH IN HEIGHT OF THREE SUGARCANE VARIETIES 71 DAYS AFTER TREATMENT

Variety	Treatment		Height to first visible dewlap (cm) Replication			Average
	Lb. a.i./acre 2/		1	2	3	
H 50-7209	Control		44.8 1/	35.7	40.4	40.3
	Diuron	9	52.6	42.5	50.9	48.6
	Ametryne	9	52.0	50.6	48.3	50.3
H 57-5174	Control		54.1	55.9	45.3	51.7
	Diuron	9	54.4	55.7	51.7	53.9
	Ametryne	9	49.3	54.4	53.1	52.2
H 53-263	Control		36.5	37.9	37.8	37.4
	Diuron	9	36.3	34.0	33.3	34.5
	Ametryne	9	25.8	22.0	33.4	27.0

Analysis of Variance

Source of variation	DF	MS
Replication	2	8.5
Variety	2	907.1**
Treatment	2	19.2
V X T	4	78.1*
Error	16	15.6
Total	26	

C.V. = 9.6

1/ An average of 12 measurements.

2/ 4 Lb./acre pre-plus 5 lb./acre post-emergence.

APPENDIX TABLE IV. EFFECT OF DIURON AND AMETRYNE ON THE GROWTH IN HEIGHT OF THREE SUGARCANE VARIETIES 92 DAYS AFTER TREATMENT

Variety	Treatment		Height to first visible dewlap (cm) Replication			Average
	Lb. a.i./acre 2/		1	2	3	
H 50-7209	Control		73.1 1/	58.3	68.9	66.8
	Diuron	9	81.5	70.4	82.6	78.2
	Ametryne	9	84.3	79.4	78.3	80.7
H 57-5174	Control		91.4	92.8	76.4	86.9
	Diuron	9	82.9	92.4	83.6	86.3
	Ametryne	9	83.5	92.4	91.6	89.2
H 53-263	Control		60.3	64.8	64.3	63.1
	Diuron	9	58.6	53.7	48.6	53.6
	Ametryne	9	40.2	35.7	51.7	42.5

Analysis of Variance

Source of variation	DF	MS
Replication	2	7.1
Variety	2	2726.8**
Treatment	2	9.0
V X T	4	240.7**
Error	16	42.6
Total	26	

C.V. = 9.0

1/ An average of 12 measurements.

2/ 4 Lb./acre pre-plus 5 lb./acre post-emergence.

APPENDIX TABLE V. EFFECT OF DIURON AND AMETRYNE ON THE GROWTH IN HEIGHT OF THREE SUGARCANE VARIETIES AT 138 DAYS AFTER TREATMENT

			Height to first visible dewlap (cm)			
Variety	Treatment		Replication			Average
	Lb. a.i./acre 2/		1	2	3	
H 50-7209	Control		134.0 1/	114.7	133.5	127.4
	Diuron	9	150.8	133.5	147.7	144.0
	Ametryne	9	151.4	144.9	136.9	144.4
H 57-5174	Control		160.3	157.7	135.2	151.0
	Diuron	9	148.5	157.7	149.3	151.8
	Ametryne	9	153.7	162.5	162.9	159.9
H 53-263	Control		123.1	131.7	129.5	128.1
	Diuron	9	107.1	105.0	95.8	102.6
	Ametryne	9	91.5	89.0	105.0	95.1

Analysis of Variance

Source of variation	DF	MS
Replication	2	
Variety	2	4826.5**
Treatment	2	19.5
V X T	4	612.68**
Error	16	76.37
Total	26	

C.V. = 6.5

1/ An average of 12 measurements.

2/ 4 Lb./acre pre-plus 5 lb./acre post-emergence.

APPENDIX TABLE VI. EFFECT OF DIURON AND AMETRYNE ON THE GROWTH IN DIAMETER OF THREE SUGARCANE VARIETIES AT 138 DAYS AFTER TREATMENT (CM)

Variety	Treatment		Replication			Average
	Lb. a.i./acre[2/]		1	2	3	
H 50-7209	Control		2.92[1/]	2.81	2.90	2.876
	Diuron	9	2.98	2.87	3.01	2.953
	Ametryne	9	2.95	2.98	2.90	2.943
H 57-5174	Control		2.76	2.70	2.58	2.680
	Diuron	9	2.55	2.62	2.52	2.563
	Ametryne	9	2.71	2.75	2.90	2.786
H 53-263	Control		3.01	3.09	2.97	3.023
	Diuron	9	2.78	2.66	2.40	2.613
	Ametryne	9	2.65	2.75	2.94	2.780

Analysis of Variance

Source of variation	DF	MS
Replication	2	
Varieties	2	.14**
Treatment	2	.06*
V X T	4	.05*
Error	16	.01
Total	26	

C.V. = 3.0

1/ An average of 12 measurements.

2/ 4 Lb./acre pre-plus 5 lb./acre post-emergence.

APPENDIX TABLE VII. EFFECT OF DIURON AND AMETRYNE ON THE NUMBER OF STALKS OF THREE SUGARCANE VARIETIES AT 188 DAYS AFTER TREATMENT

Variety	Treatment		Replication			Average
	Lb. a.i./acre[2/]		1	2	3	
H 50-7209	Control		8.7[1/]	7.4	9.0	8.36
	Diuron	9	8.7	8.2	6.8	7.90
	Ametryne	9	7.1	8.1	7.6	7.60
H 57-5174	Control		12.2	11.6	9.8	12.20
	Diuron	9	11.0	10.7	10.2	10.63
	Ametryne	9	12.2	11.8	9.5	11.16
H 53-263	Control		10.6	10.5	10.5	10.53
	Diuron	9	7.0	7.6	5.8	6.80
	Ametryne	9	7.5	8.8	7.6	7.96

Analysis of Variance

Source of Variation	DF	MS
Replication	2	2.42
Variety	2	24.15**
Treatment	2	6.03**
V X T	4	2.84*
Error	16	.60
Total	26	

C.V. = 8.4

1/ An average of 12 measurements.

2/ 4 Lb./acre pre-plus 5 lb./acre post-emergence.

APPENDIX TABLE VIII. EFFECT OF DIURON ON THE GROWTH OF VARIETY H 53-263 IN NUTRIENT SOLUTION 8 DAYS AFTER TREATMENT

Treatment (ppm diuron)	Gain in Fresh Weight (grams) Replication 1/			
	1	2	3	Average
0.0	10.40	7.30	4.10	7.26
0.1	9.00	5.00	2.30	5.43
0.3	6.10	4.60	1.90	4.20
0.4	5.00	3.00	1.70	3.23
0.5	3.80	4.00	0.00	2.60
0.7	1.50	1.20	.50	1.06

APPENDIX TABLE IX. EFFECT OF DIURON ON THE GROWTH OF VARIETY H 50-7209 IN NUTRIENT SOLUTION 8 DAYS AFTER TREATMENT

Treatment (ppm diuron)	Gain in Fresh Weight (grams) Replication 1/			
	1	2	3	Average
0.0	27.4	11.8	14.9	18.03
1.0	9.3	10.6	11.6	10.5
2.0	6.5	5.6	8.3	6.8
3.0	6.0	10.0	5.9	7.3
4.0	10.7	6.0	5.1	7.2
5.0	5.5	4.0	5.2	4.9

1/ The plants were separated into replications based on initial size. Replications 1, 2, and 3 contained large, medium, and small plants, respectively. Comparisons of treatment effects should be made within a given replication.

APPENDIX TABLE X. EFFECT OF MONOMETHYLDIURON ON VARIETY H 53-263 IN NUTRIENT SOLUTION 8 DAYS AFTER TREATMENT

Treatment (ppm diuron)	Gain in Fresh Weight (grams) Replication			
	1	2	3	Average
0.0	15.6	12.3	5.6	11.2
0.5	11.6	7.5	7.1	8.7
0.7	11.5	5.1	3.2	6.5
0.9	11.2	4.4	1.2	5.6
1.2	3.9	0.7	2.9	2.5

APPENDIX TABLE XI. EFFECT OF DIURON ON THE GROWTH OF 4 VARIETIES OF SUGARCANE IN SAND CULTURE

The length of the longest leaf 41 days after treatment with diuron (cm)

Variety	Treatment	Replication 1	2	3	Average	
H 50-7209	Control	102.2 1/	103.8	146.0	117.4 2/	ab
	Diuron (3 ppm)	140.5	147.3	149.0	145.8	a
H 44-3098	Control	123.8	99.8	99.3	107.6	b
	Diuron (3 ppm)	82.8	115.5	80.5	91.6	b
H 57-5174	Control	132.0	133.3	69.3	111.3	ab
	Diuron (3 ppm)	77.0	99.5	92.8	89.8	b
H 53-263	Control	136.0	98.5	73.3	102.6	b
	Diuron (3 ppm)	84.7	79.0	72.0	78.6	b

1/ Average of 4 plants.

2/ Means followed by the same letter are not significantly different. Duncan's multiple range test (5% level).

APPENDIX TABLE XII. EFFECT OF DIURON ON THE DRY WEIGHT OF FOUR VARIETIES OF SUGARCANE IN SAND CULTURE

The dry weights of sugarcane tops 41 days after treatment with diuron (cm)

Variety	Treatment	Replication 1	2	3	Average	
H 50-7209	Control	14.76 1/	13.06	34.23	20.68 2/	ab
	Diuron (3 ppm)	16.01	18.65	16.84	17.19	bcd
H 44-3098	Control	14.63	9.82	9.81	11.42	bcde
	Diuron (3 ppm)	4.55	7.04	4.68	5.48	efg
H 57-5174	Control	19.31	23.19	9.63	17.38	bc
	Diuron (3 ppm)	3.80	6.55	4.65	5.00	efg
H 53-263	Control	37.69	20.04	29.27	29.00	a
	Diuron (3 ppm)	7.02	6.07	5.07	6.05	ef

1/ Weight of all plants in pot exclusive of roots (4 plants).

2/ Means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

APPENDIX TABLE XIII. EFFECT OF DIURON ON THE TILLERING OF FOUR VARIETIES OF SUGARCANE IN SAND CULTURE

The number of tillers per plot 41 days after treatment with diuron[1/]

Variety	Treatment	Replication 1	2	3	Average
H 50-7209	Control	2	1	7	3.3
	Diuron (3 ppm)	0	2	2	1.3
H 44-3098	Control	5	5	5	5.0
	Diuron (3 ppm)	1	0	2	1.0
H 57-5174	Control	7	7	7	7.0
	Diuron (3 ppm)	0	0	0	0.0
H 53-263	Control	10	10	2	7.3
	Diuron (3 ppm)	0	0	0	0.0

1/ Average of four plants.

APPENDIX TABLE XIV. EFFECT OF DIURON ON THE GROWTH IN HEIGHT OF THREE SUGARCANE VARIETIES IN SAND CULTURE

Height of sugarcane to first visible dewlap 43 days after treatment (cm)

Variety	Treatment	Replication 1	2	3	Average	
H 50-7209	Control	30.7 1/	28.5	28.0	29.1 2/	a
	Diuron (1 ppm)	32.5	27.5	27.3	29.1	a
	Diuron (3 ppm)	27.5	30.3	21.3	26.3	ab
H 57-5174	Control	21.8	20.2	21.3	21.1	c
	Diuron (1 ppm)	17.7	14.5	14.5	15.7	d
	Diuron (3 ppm)	10.0	8.3	10.5	9.6	f
H 53-263	Control	23.0	25.0	20.5	22.8	bc
	Diuron (1 ppm)	17.3	13.5	14.5	15.1	de
	Diuron (3 ppm)	12.3	14.0	12.0	12.7	df

1/ Average of two plants.

2/ Means with same letter are not significantly different at the 5% level (Duncan's multiple range test).

APPENDIX TABLE XV. EFFECT OF DIURON ON THE GROWTH IN HEIGHT OF THREE SUGARCANE VARIETIES IN SAND CULTURE

Height of sugarcane to first visible dewlap 33 days after treatment (cm)

Variety	Treatment	Replication 1	2	3	Average	
H 50-7209	Control	25.5 1/	22.5	22.5	23.5 2/	a
	Diuron (1 ppm)	26.5	20.5	17.5	21.5	a
	Diuron (3 ppm)	19.5	24.0	17.0	20.2	abc
H 57-5174	Control	18.0	17.5	17.5	17.6	cde
	Diuron (1 ppm)	12.5	12.0	12.0	12.16	fgh
	Diuron (3 ppm)	8.5	8.5	10.0	9.0	gh
H 53-263	Control	19.0	20.5	20.5	20.0	abcd
	Diuron (1 ppm)	17.0	13.5	15.0	15.2	ef
	Diuron (3 ppm)	12.0	14.0	12.0	12.7	fg

1/ Average value for two plants.

2/ Means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

APPENDIX TABLE XVI. DISTRIBUTION OF RADIOACTIVITY ON TLC STRIPS SPOTTED WITH EXTRACTS OF ROOTS, STEMS AND LEAVES (1 WEEK)

	Percent of Total Radioactivity on TLC Plates[1]					
	Rf Values[2]					
	0	.05-.25	.25-.45	.45-.65	.65-.85	.85-1.0
Roots						
H 50-7209	4.0	9.6	47.5	31.2	4.0	3.5
H 53-263	4.3	5.6	22.6	48.3	11.4	7.8
Stems						
H 50-7209	15.5	15.0	38.2	18.5	8.0	4.8
H 53-263	2.8	3.7	28.1	35.3	5.0	24.5
Leaves						
H 50-7209	18.5	6.4	58.0	11.7	4.8	0
H 53-263	5.3	1.1	23.7	63.7	3.3	3.0

1/ Average of three replications.

2/ Rf values:

Diuron	.54
Monomethyldiuron	.43
Demethylated diuron	.29

APPENDIX TABLE XVII. DISTRIBUTION OF RADIOACTIVITY ON TLC STRIPS SPOTTED WITH EXTRACTS OF ROOTS, STEMS AND LEAVES (3 WEEKS)

	Percent of Total Radioactivity on TLC Plates[1/]					
	Rf Values[2/]					
	0	.05-.25	.25-.45	.45-.65	.65-.85	.85-1.0
Roots						
H 50-7209	8.7	5.2	45.5	22.5	12.5	5.5
H 53-263	8.3	4.5	35.9	41.8	8.1	1.1
Stems						
H 50-7209	17.5	12.1	41.0	11.0	11.7	4.6
H 53-263	3.5	11.1	27.5	19.3	35.7	2.9
Leaves						
H 50-7209	18.3	15.9	42.5	14.5	7.9	.7
H 53-263	3.0	4.0	27.8	58.3	5.5	1.3

1/ Average of 4 replications (H 50-7209) -- 3 replications (H 53-263).

2/ Rf values:

Diuron	.54
Monomethyldiuron	.43
Demethylated diuron	.29